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The biology of *Gynnidomorpha permixtana* (Lepidoptera, Tortricidae) on *Sagittaria trifolia* L. (Alismataceae) in paddy fields in Iran

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Abstract. While testing the efficacy of herbicides on paddy weeds at the Rice Research Institute of Iran (RRII) in 2008, we encountered the failure of arrowhead (*Sagittaria sagittifolia* L., Alismataceae) seeds to germinate. Detailed investigation revealed physical damage of seeds caused by the larvae of *Gynnidomorpha permixtana* (Denis & Schiffermüller, 1775) (Tortricidae, Tortricinae, Cochylina). Further studies showed that larvae feed on the seeds and flowers of the host plant and destroy the achenes. Under laboratory conditions *G. permixtana* required 23–30 days to complete its life cycle. Arrowhead is a new host record for this moth species; furthermore, this is the first detailed record of a tortricid feeding on this plant.

Introduction

Arrowhead (*Sagittaria sagittifolia* L.; Alismataceae) is a perennial weed that is present throughout the rice growing areas of eastern Asia (Naylor 1996). It is a major weed pest of paddy rice in Iran (Mohammadsharifi 2000). Arrowhead is difficult to control due to herbicide tolerance and season-long emergence (Kwon 1993). In Korea and Japan arrowhead is ranked as the most important weed in paddy fields (Itoh and Miyahara 1988; Kwon 1993; Chung et al. 1998).

Until now eighty-two species of Cochylini from twelve genera have been recorded from Iran and these are the following: *Aethes* Billberg, 1820; *Agapeta* Hübner, 1822; *Ceratoxanthis* Razowski, 1960; *Cochylidia* Obraztsov, 1956; *Cochylimorpha* Razowski, 1960; *Cochylis* Treitschke, 1829; *Diceratura* Djakonov, 1929; *Eugnosta* Hübner, 1825; *Fulvoclysia* Obraztsov, 1943; *Gynnidomorpha* Turner, 1916; *Phalonidia* Le Marchand, 1933 and *Phtheochroa* Stephens, 1829 (Alipanah 2009).

The genus *Gynnidomorpha* Turner, 1916, assigned to the subtribe Cochylina (Tortricidae: Tortricinae), includes 17 species that are recorded from the Holarctic, Oriental, and Australian regions, with greatest species richness documented from the Palaearctic region (Sun and Li 2013). *Gynnidomorpha permixtana* (Fig. 1) is a trans-Palaearctic species, occurring from the British Isles to the Ural Mts., Afghanistan, Mongolia, China (Razowski 2002), Korea (Byun and Li 2006) and Iran (Alipanah 2009). Host plants previously reported for this moth include *Alisma* sp. (Alismataceae), *Gentiana* sp. (Gentianaceae), *Euphrasia* sp. and *Pedicularis* sp. (Scrophulariaceae) from Japan; *Alisma orientale* (Sam.) Juzepcz, *Euphrasia pectinata* M. Tenore.,

Figures 1–2. *Gynnidomorpha permixtana* morphological characteristics: **1.** Adult; **2.** Genitalia slide (Photo: L. Aarvik).

Pedicularis L., and *Gentiana lutea* L. from China; and *Euphrasia*, *Odontites* (Orobanchaceae), *Rhinanthus alectorolophus* (Scrophulariaceae), and *Pedicularis* from Europe (Table 1) (Disque 1908; Razowski 1970; Bradley et al. 1973; Kawabe 1982; Liu and Li 2002) .

Material and methods

Rearing. Laboratory colonies were established by collecting pupae and larvae from arrowhead seeds showing symptoms of damage. The samples were taken from experimental paddy fields at the Rice Research Institute of Iran (RRII) (N 37°12′22.2″, E 049°38′40.7″, 80 m) from 2008 to 2012. Samples were collected from the third week of August to the third week of September each year. Pupae and larvae were kept in rearing containers 12 cm diameter and 24 cm in height. Each container was furnished with about 50 grams of arrowhead seeds.

After emergence males and females were moved for mating and placed in transparent plastic containers 12 cm diameter and 14 cm height that contained 50 grams of fresh arrowhead seeds. Once a female laid eggs, no additional adults were placed in the container, and the colony continued its development naturally. After two weeks about 50 grams of fresh arrowhead seeds were added to each container. Colonies were inspected daily, and all activities, including egg hatching, larval feeding period, pupation, and emergence of adults were recorded. A second generation was produced using adults of the first generation's egg masses that were transferred into mating containers. About four generations were produced each year.

Identification. Twenty samples were used for identification each year. Morphological terminology follows Razowski (1987). Preliminary identification was conducted in the RRII entomology laboratory based on wing venation (Sun and Li 2013), shape of the labial palpus, and other morphological aspects (Byun et al. 2007). Dissection of the genitalia (Fig. 2) of an adult male in the Natural History Museum of University of Oslo, confirmed the identity of the species as *Gynnidomorpha permixtana* (Denis & Schiffermüller, 1775).

Table 1. Cited from Tortricid.net, host plant data base in addition to Japanese and Chinese data.

Host plant	Plant family	Herbivore	Subfamily	Georegion	Location
Alisma plantago-aquatica L.	Alismataceae	*Gynnidomorpha alismana* (Ragonot) (as *Phalonidia*)	Tortricinae	Bradley et al. 1973	Europe
Alisma plantago-aquatica L.	Alismataceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *mussehilana*)	Tortricinae	Disque 1908	Europe
Alisma plantago-aquatica L.	Alismataceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973	Europe
Alisma plantago-aquatica L.	Alismataceae	*Gynnidomorpha vectisana* (Humphreys & Westwood) (as *geyeriana*)	Tortricinae	Disque 1908	Europe
Alisma sp.	Alismataceae	*Gynnidomorpha alismana* (Ragonot) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973	Europe
Blackstonia sp	Unknown	*Gynnidomorpha rubricana* (Peyerimhoff)	Tortricinae	Trematerra and Baldizzone 2004	Europe Croatia
Butomus umbellatus L.	Butomaceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller)	Tortricinae	Disque 1908; Bradley et al. 1973	Europe
Euphrasia sp.	Scrophulariaceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973; Kawabe 1982; Liu and Li 2002	Europe, Japan and China
Gentiana lutea L.	Gentianaceae	*Gynnidomorpha permixtana* (Denis and Schiffermueller) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973; Kawabe 1982; Liu and Li 2002	Europe, Japan and China
Marticaria recutita L.	Asteraceae	*Gynnidomorpha luridana* (Greyson) (as *Phalonidia*)	Tortricinae	Bradley et al. 1973	Europe
Marticaria recutita L.	Asteraceae	*Gynnidomorpha luridana* (Gregson)	Tortricinae	Trematerra and Baldizzone 2004	Europe Croatia
Menyanthes trifoliata L.	Menyanthaceae	*Gynnidomorpha minimana* (Caradja) (as *Phalonidia*)	Tortricinae	Bradley et al. 1973	Europe
Pedicularis palustris L.	Scrophulariaceae	*Gynnidomorpha minimana* (Caradja) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973	Europe
Pedicularis sp.	Scrophulariaceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973, Kawabe 1982; Liu and Li 2002	Europe, Japan and China
Plantago maritima L.	Plantaginaceae	*Gynnidomorpha vectisana* (Humphreys & Westwood) (as *Phalonidia*)	Tortricinae	Razowski 1970	Europe
Rhinanthus minor L. (as *Alectorolophus*)	Scrophulariaceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *mussehilana*)	Tortricinae	Disque 1908	Europe
Rhinanthus sp.	Scrophulariaceae	*Gynnidomorpha permixtana* (Denis & Schiffermueller) (as *Phalonidia*)	Tortricinae	Razowski 1970; Bradley et al. 1973;	Europe
Salicornia sp.	Chenopodiaceae	*Gynnidomorpha vectisana* (Humphreys & Westwood) (as *Phalonidia*)	Tortricinae	Razowski 1970	Europe
Triglochin maritima L.	Juncaginaceae	*Gynnidomorpha vectisana* (Humphreys & Westwood) (as *Phalonidia*)	Tortricinae	Bradley et al. 1973	Europe

Field observation. Field studies were based on collecting by light traps, examination of arrowhead seeds and flowers that showed signs of injury, and inspection of other host plants (such as *Alisma* sp.) for characteristic signs of damage. At least three paddy fields with heavy infestations were inspected each year.

Results

Eggs. Eggs are small (ca. 0.5 mm diameter), round, white or opaque (Fig. 3), and are laid singly or in groups of 2–3 on a smooth surface of the host plant. In the laboratory, eggs hatched after an average of 3–4 days incubation at 27–31 °C. However, the incubation period may be correlated with temperature because eggs held at < 25 °C showed a conspicuous increase in the incubation period (6–9 days at 21–25 °C).

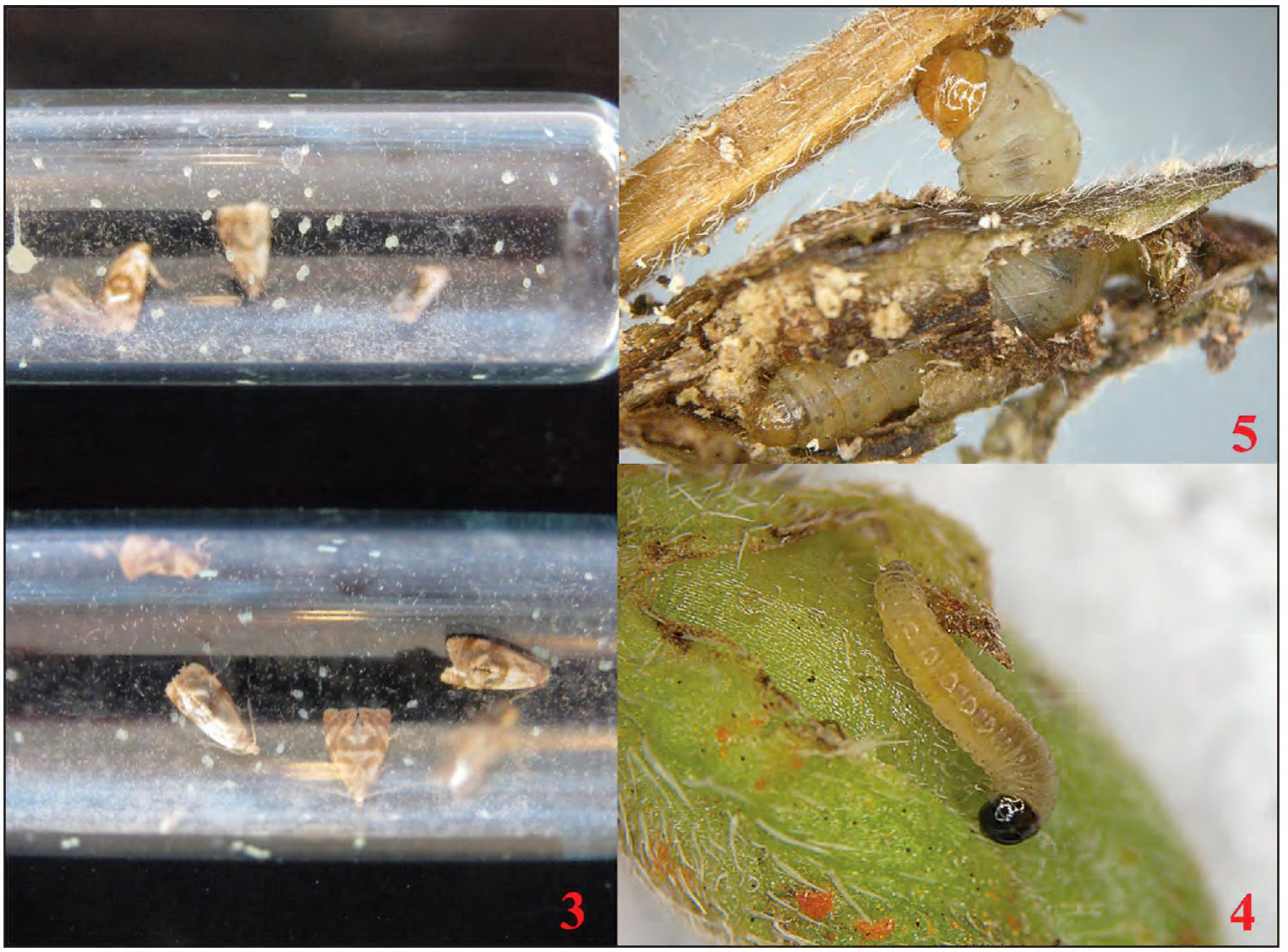
Larva. Newly hatched larvae (Fig. 4) are off-white, about 1–1.5 mm long, and have a dark brown to nearly black head. Mature larvae (Fig. 5) are 7–10 mm long, their color depending on their diet. If fed on fresh seeds, they are pale green to medium green; if fed on dry seeds they are yellow-brown to straw colored. The head is light yellow-brown, and the 1st segment (prothorax) is light greenish-brown. Larvae are figured by Beavan and Heckford 2012. In the laboratory (at 27–31 °C) larvae require 4–5 instars that last about 12–15 days. Early instars do not leave the seeds, but the later ones do, searching for food or a pupation site.

Pupa. Pupation usually occurs inside seeds of the food plant or in shelters made by larvae from other available material. Pupae (Fig. 6) are 6–7 mm long, and turn from greenish-brown to brown as they mature. In the laboratory (at 27–31 °C) the pupal period lasts 7–9 days. Upon adult eclosure, the exuvium is partly extruded from the shelter (Figs 7, 8).

Symptoms of damage on the food plant. Larvae feed on flowers and seeds of arrowhead. They disperse after hatching and tunnel through seeds. The entrance holes of the tunnels are inconspicuous; therefore initially there is no external evidence of larval feeding. After about one week the entrance holes become marked by a protruding mass of frass which has the appearance of grains of sand and silk that accumulate on the surface of the achenes (Fig. 9). One to several larvae may be present in a single fruit. Young larvae feed inside flower buds which are destroyed and hence abort. Flower buds and seeds dry up and disintegrate within about two weeks after infestation, but seeds that contain pupae and older instars remain on green flower tops (Fig. 10). In this way larvae destroy most of the achenes or cause severe injury to them. In addition, those few achenes that remain uninjured decompose after a few weeks and disintegrate because larvae spin them into silk webs together with injured seeds (Fig. 11). In some cases the remaining seed heads contain only frass and silk webs because the larvae have destroyed all achenes and their germplasm. At this stage frass and destroyed achenes form sawdust-like masses which fall to the ground.

Discussion

Numerous host plants have been recorded for Cochylini, but most of them belong to Asteraceae, and some of the Cochylini genera (e.g. *Aethes* and *Cochylis*) mainly feed on this family (Razowski 1970; Trematerra and Baldizzone 2004; Fazekas 2008; Sun and Li 2013). Worldwide, there are few host plant records for *Gynnidomorpha* spp. and the host plant of many species is unknown. However, for some species of *Gynnidomorpha*, in addition to Asteraceae, species of Alismataceae, Butomaceae, Scrophulariaceae, Gentianaceae, Menyanthaceae, Plantaginaceae, Chenopodiaceae and Juncaginaceae have been recorded as food plants (Table 1) (Disque 1908;

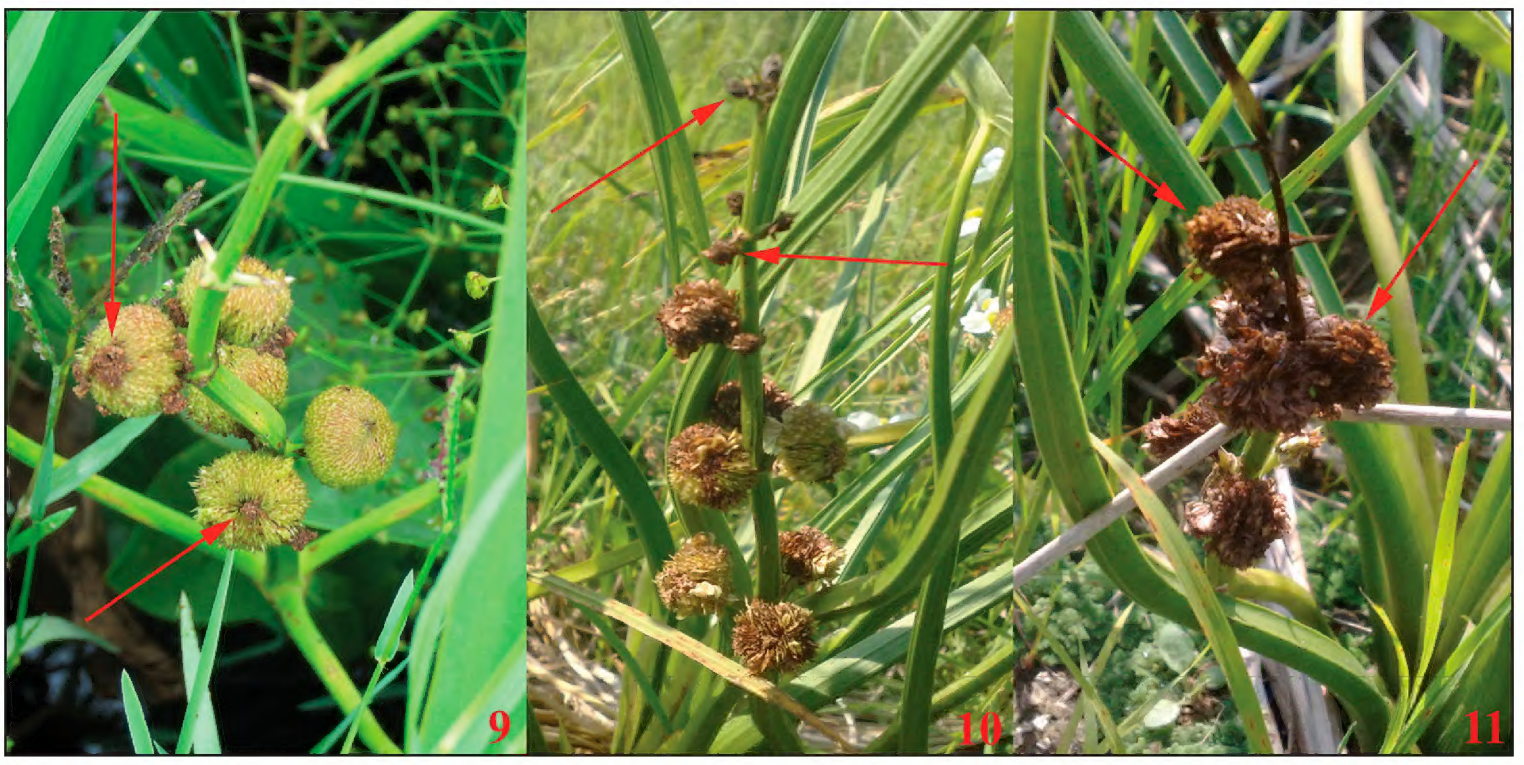


Figures 3–5. Immature stages of *G. permixtana*. **3.** Eggs; **4.** Early instar larva; **5.** Final instar larva (Photo: **3.** A. Farahpour; **4, 5.** Beavan & Heckford).

Razowski 1970; Bradley et al. 1973). Based on literature records some of the *Gynnidomorpha* species, such as *G. alismana* and *G. rubricana*, seem to be monophagous and feed on only one genus or species. Other species are oligophagous and have a range of food plants. Among them *G. permixtana* has the largest range of host plants.

Kaltenbach (1856) stated that he had reared a *Cochylis* larva from *Sagittaria sagittifolia* L. but he did not mention the species (Beavan and Heckford 2012). In China a *Sagittaria* sp. has been reported as the food plant of *G. mesotypa* (Sun and Li 2013) but *G. mesotypa* has not been reported from Iran. Laboratory rearings confirmed that *G. permixtana* is an internal feeder of arrowhead seeds and flower buds. Westbury (2004) reported that *G. permixtana* is a seed predator of *Rhinanthus minor*; Bradley et al. (1973) stated that *G. permixtana* larvae feed in the flower heads, seeds and stems of *Rhinanthus*. Organisms that feed internally, particularly on meristematic tissues, often affect the plant more seriously (Center et al. 2002). Beavan and Heckford (2012) described *G. permixtana* behavior on *Rhinanthus minor*. They stated that each larva fed entirely within one seed-capsule and sometimes a larva would spin two seed-capsules together and apparently transfer from one to the other and there would never be more than one larva in any one capsule. They suggested that because each seed-capsule was no longer than 5 mm, at that stage each larva fed entirely within one seed-capsule. They also reported that the seed-capsules with larvae sometimes had some dark discoloration on the outside but otherwise there were no outward visible signs that they were inhabited and that full-grown larvae had habit of wander-

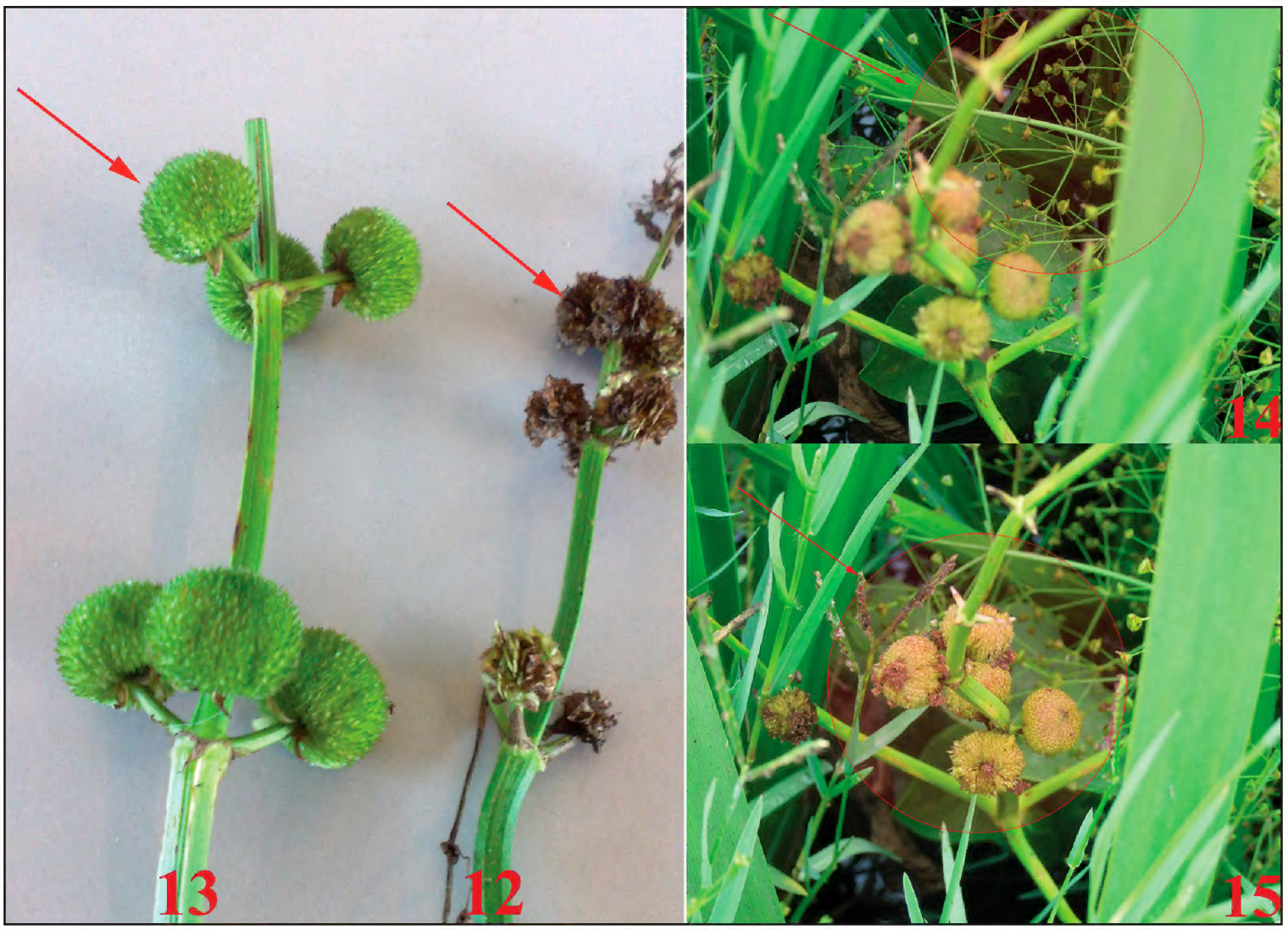
Figures 6–8. Maturing stages of *G. permixtana*: **6.** Pupa; **7.** Newly emerged adult with partly exuded exuvium; **8.** Living adult (Photo: A. Farahpour).



Figures 9–11. Damage symptoms on food plant. **9.** About one week after hatching, arrows showing marked entrance holes; **10.** About two weeks after hatching, arrows showing dried up flower buds; **11.** About three weeks after hatching, arrows showing dried up fruits (Photo: A. Farahpour).

ing about the containers which contained infested fruits. Each fruit of *Sagittaria sagittifolia* L. contained more than one larva and as already mentioned there was no visible signs of infestation in the first stage. However, after a few days symptoms of infestation became visible. Other habits, such as spinning achenes and wandering about the infested sites, were apparently the same.

Sagittaria spp. emerge at 16 °C to 17 °C (Noda 1977). Under the climatic conditions of northern Iran the activity period is from late June to late November. If there is suitable temperature and weather in November and December, they will continue their activity until late December. Results of field inspections showed that *G. permixtana* was active from the beginning of July until late November. Sun and Li (2013) and Razowski (2009) reported that in Europe the *Gynnidomorpha* species are usually bivoltine and occur in various habitats, but many of them are restricted to humid or even aquatic ones. Northern Iran is humid and paddy field has aquatic



Figures 12–15. Infested fruits compared with uninfested fruits at the same age. **12.** Infested fruits; **13.** Uninfested fruits (Photo: A. Farahpour). **14–15.** *Sagittaria sagittifolia* and *Alisma* sp. seeds from same spot. **14.** *Alisma* sp. seeds without infestation symptoms; **15.** *Sagittaria sagittifolia* seeds with infestation symptoms (Photo: A. Farahpour).

habitat so environmental conditions are suitable for this species. Also, in the laboratory as long as its food plant was available and temperature was optimal, it continued its activity. Since its food plant became limited each year during the winter, we are uncertain about its facultative diapause. However, food source and environmental conditions could affect voltinism. The synchronization between *G. permixtana* and arrowhead seems to be strong, because in 2010 when the weather was mild in November and December, this species (*G. permixtana*) continued its activity until late December. Close synchrony between an insect and the resource dynamics of its host can lead to increased insect densities, whereas consequences of asynchronization between insect and plant include reduced insect density (Turgeon 1986). In addition to various bottom-up effects, the synchronization of herbivores with their host plant phenology determines quality and quantity of food resources and affects the preference–performance linkage and abundance of herbivores (Yukawa 2000). Therefore, this close synchronization with host plant could be an important and beneficial factor for *G. permixtana*.

When injured seeds of infested fruits (Fig. 12) were inspected in the laboratory and compared with uninfested fruits (Fig. 13), physical damage caused by larval feeding was severe. Since fruits consist of multiple achenes, each larva is capable of destroying more than one seed. Seeds are partially or completely consumed. Furthermore, as feeding progresses, the seeds become bound together with coarse frass and webbing. In arrowheads, sexual reproduction via

seeds is better for establishing new populations because of their small size and ability to disperse. Being small and buoyant, the seeds are capable of 'rafting' to suitable sites on relatively light currents, gathering in still pools and river bends. This mechanism can bring about the redistribution of genes along and among waterways (Broadhurst and Chong 2011). When they are bound together they are unable to raft and/or disperse.

We were unable to find symptoms of damage and biology of *G. permixtana* on other food plants such as *Alisma* sp. Despite the fact that *Alisma* is common in the investigated fields (Fig. 14), we could not find any trace of *G. permixtana* activity on that plant (Fig. 15), and all our attempts to rear a generation on other food plants in the laboratory failed.

The importance of plant-feeding insects in the dynamics of aquatic and wetland ecosystems is often unappreciated. This is most often due to the unfamiliarity of resource managers, scientists, and others with the plant-feeding insects that are present in these ecosystems. The purpose of this study was mainly to facilitate the recognition of *G. permixtana*, and review its biology and the symptoms of damage that it causes on arrowhead and therefore statistical methods were not employed. Determination of beneficial factors of *G. permixtana* as a biological control agent needs more biological and statistical studies. The information that we discovered about this species may represent an important step in this direction.

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